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GENETIC AND PHENOTYPIC CATALOG OF NATIVE RESIDENT TROUT OF THE INTERIOR COLUMBIA RIVER BASIN: POPULATIONS IN THE WENATCHEE, ENTIAT, LAKE CHELAN, & METHOW RIVER DRAINAGES

FY 2001 Report

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GENETIC AND PHENOTYPIC CATALOG OF NATIVE RESIDENT TROUT OF THE INTERIOR COLUMBIA RIVER BASIN

FY-2001 REPORT

POPULATIONS IN THE WENATCHEE, ENTIAT, LAKE CHELAN, AND METHOW RIVER DRAINAGES

Report Prepared For

NORTHWEST POWER PLANNING COUNCIL
UPPER COLUMBIA BASIN FISH AND WILDLIFE PROGRAM

BPA Contract Number 00004575

By

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GENETIC AND PHENOTYPE CATALOG OF NATIVE RESIDENT TROUT OF THE INTERIOR COLUMBIA RIVER BASIN

FY-2001 REPORT: POPULATIONS IN THE WENATCHEE, ENTIAT, LAKE CHELAN, AND METHOW RIVER DRAINAGES

EXECUTIVE SUMMARY

In fiscal year 2001, nonlethal fin tissues were collected for genetic analysis from fourteen stream trout populations (nine cutthroat populations and five rainbow populations) residing in headwater reaches of Wenatchee, Entiat, Lake Chelan, and Methow River tributaries. Four of these collections were made collaboratively with the U. S. Forest Service; we provided photography and they assumed responsibility for the genetic analysis. Using a portable aquarium, we photographed representative specimens of each population for a color catalog of appearance phenotypes. Analysis of paired interspersed nuclear DNA elements (PINEs) was used to characterize each population as to subspecies and level of hybridization, and a genetic purity rating was assigned to each using a modification of the Binns system originally developed in Wyoming to gauge the genetic purity of interior cutthroat trout populations.

Six of the cutthroat trout populations we collected were genetically pure westslope cutthroat trout *Oncorhynchus clarki lewisi*. The other two populations were good examples of *lewisi* as well, appearance-wise, but these populations contained individuals hybridized with rainbow trout, ranging from 11 percent to 29 percent of the individuals in the population. Owing to a limitation of the PINE technique, we were unable to state whether the rainbow trout contribution to these hybrids was from the Columbia River redband subspecies *O. mykiss gairdneri*, which occurs as the native rainbow subspecies in the lower reaches of most if not all of the stream systems, or from the coastal rainbow subspecies *O. m. irideus* which has been widely stocked in these basins. In terms of the Binns purity rating, only two of the six genetically pure *lewisi* populations were given A–ratings (no hybridization and no history of cutthroat trout stocking anywhere in the system). These were Snowy Creek (Wenatchee drainage) and State Creek (Lake Chelan drainage). The other pure *lewisi* populations were rated B because they occur in streams where hatchery cutthroat trout have been stocked in the past.

Only one of the five rainbow trout populations examined in this study (upper Icicle Creek, Wenatchee drainage) was given the A rating for genetic purity. This population appears to be a good representative of the interior Columbia River redband subspecies *O. mykiss gairdneri*.

Our discovery of two A–populations (no hybrids and no history of stocking) of westslope cutthroat trout in headwater tributaries of these basins lends additional weight to earlier suggestions that the range of *O. c. lewisi* extends into central Washington State westward to the Cascade crest. It also suggests that the evolutionarily younger and later-invading interior rainbow trout has not completely displaced native cutthroat from this range, especially not from the uppermost reaches of trout-bearing waters.

The precise locations of our collection sites as well as maps, site descriptions, photographs, and habitat conditions as we found them are given in the report. Although not a complete inventory by any means, this information should be of great value to managers in coming years for stewardship of the native resident trout populations, especially in the face of the potential listing of the westslope cutthroat trout under the U. S. Endangered Species Act.

INTRODUCTION

The 1994 Fish and Wildlife Program of the Northwest Power Planning Council specifies the recovery and preservation of population health of native resident fishes of the Columbia River Basin. Among the native resident species of concern are interior rainbow trout of the Columbia River redband subspecies *Oncorhynchus mykiss gairdneri*[1] and westslope cutthroat trout *O. clarki lewisi*. The westslope cutthroat trout has been petitioned for listing under the U. S. Endangered Species Act (American Wildlands et al. 1997).

Before at-risk populations can be protected, their presence and status must be established. Where introgression from introduced species is a concern, as in the case of both westslope cutthroat trout and redband rainbow trout, genetic issues must be addressed as well. As is true with native trout elsewhere in the western United States (Behnke 1992), most of the remaining pure populations of these species in the Columbia River Basin are in relatively remote headwater reaches.

The objective of this project was to photo-document upper Columbia Basin native resident trout populations in Washington, and to ascertain their species or subspecies identity and relative genetic purity using a nonlethal DNA technique. FY-2001 was year three (and final year) of a project in which we conducted field visits to remote locations to seek out and catalog these populations. In FY-2001 we worked in collaboration with the Wenatchee National Forest to catalog populations in the Wenatchee, Entiat, Lake Chelan, and Methow River drainages of Washington State.

THE STUDY AREA

All of our FY-2001 collection sites were located on federal lands in the Wenatchee and Okanogan National Forests. The entire study area lies within the North Cascades Ecoregion (EPA 1995, 1998). Franklin and Dyrness (1973, reprinted 1988) earlier referred to this same general area as the Northern Cascades Province. Both the terms Ecoregion and Province are used to delineate unique combinations of landscape features having distinctive terrestrial vegetation and climate. Most of this

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[1] The common and scientific names used here are those of Behnke (1992).

particular ecoregion lies between 610 and 2,134 m (2,000 to 7,000 feet) elevation, and local relief often exceeds 914 m (3,000 feet). Several higher-elevation volcanic peaks also occur here, including Mt. Baker at 3,285 m (10,778 ft), Mt. Shuksan at 2,781 m (9,124 ft), and Glacier Peak at 3,209 m (10,528 ft). This ecoregion contains more active glaciers than any other area within the lower 48 states (Post et al. 1971; Franklin and Dyrness 1973 reprinted 1988), and many ridges and peaks have glacial features. There are literally hundreds of cirques, and some peaks, ringed by cirques, have been eroded to matterhorns. Average annual precipitation varies across the ecoregion from 1,270 mm (50 inches) to 2,540 mm (100 inches). Stream density is commonly 0.009 to 0.012 km of perennial stream per ha of land area (1.5 to 2 miles per sq. mi.). Watersheds as small as 259 to 518 ha (640 to 1,280 acres or 1 to 2 sq. mi.) may support perennial streams; on the other hand, watersheds that are contained completely within the ecoregion commonly exceed 129,500 ha (320,000 acres or 500 sq. mi.). Many alpine lakes exist that were formed by glacial geomorphic processes.

The study area is largely forested. Typical tree species include Pacific silver fir, subalpine fir, Douglas-fir, mountain hemlock, western hemlock, western larch, subalpine larch, western white pine, whitebark pine, western red-cedar, and Engleman spruce (Franklin and Dyrness 1973 reprinted 1988; EPA 1998). Alpine meadows, bare rock, glaciers, and snow fields occur at the higher elevations. The Wenatchee Mountains in the southern part of the study area contain extensive serpentine outcroppings with their own unique vegetation characteristics (Franklin and Dyrness 1973 reprinted 1988). Principal land uses in the study area are forestry and recreation, with wildlife habitat also being important. Mining and prospecting (both recreational and commercial) and livestock grazing also occur in the ecoregion.

During the Pleistocene, many of the river valleys in the study area were influenced by alpine glaciers that extended down-valley for different distances (Page 1939; Barksdale 1941; Long 1951, 1989, undated), forming U-shaped valleys and leaving some tributaries in hanging valleys where they passed. V-shaped valleys and steep tributaries prevail where these alpine glaciers did not reach. Cordilleran ice straddled the Cascade crest between the Skagit and Methow drainages and extended tongues that completely occupied the Chelan and Methow valleys, coalescing with the Okanogan lobe of the ice sheet on the east (Waitt 1972; Waitt and Thorson 1983). Cordilleran ice did not extend across the divide from the Chelan valley to the Entiat (Waitt and Thorson 1983); the Entiat valley was influenced by alpine glaciation only (Long 1951, undated). What all this means is that some of our study sites would have had to be recolonized by fishes after retreat of the ice, while other sites, downstream of glacial influence or those in hanging valleys, may have served as refugia.

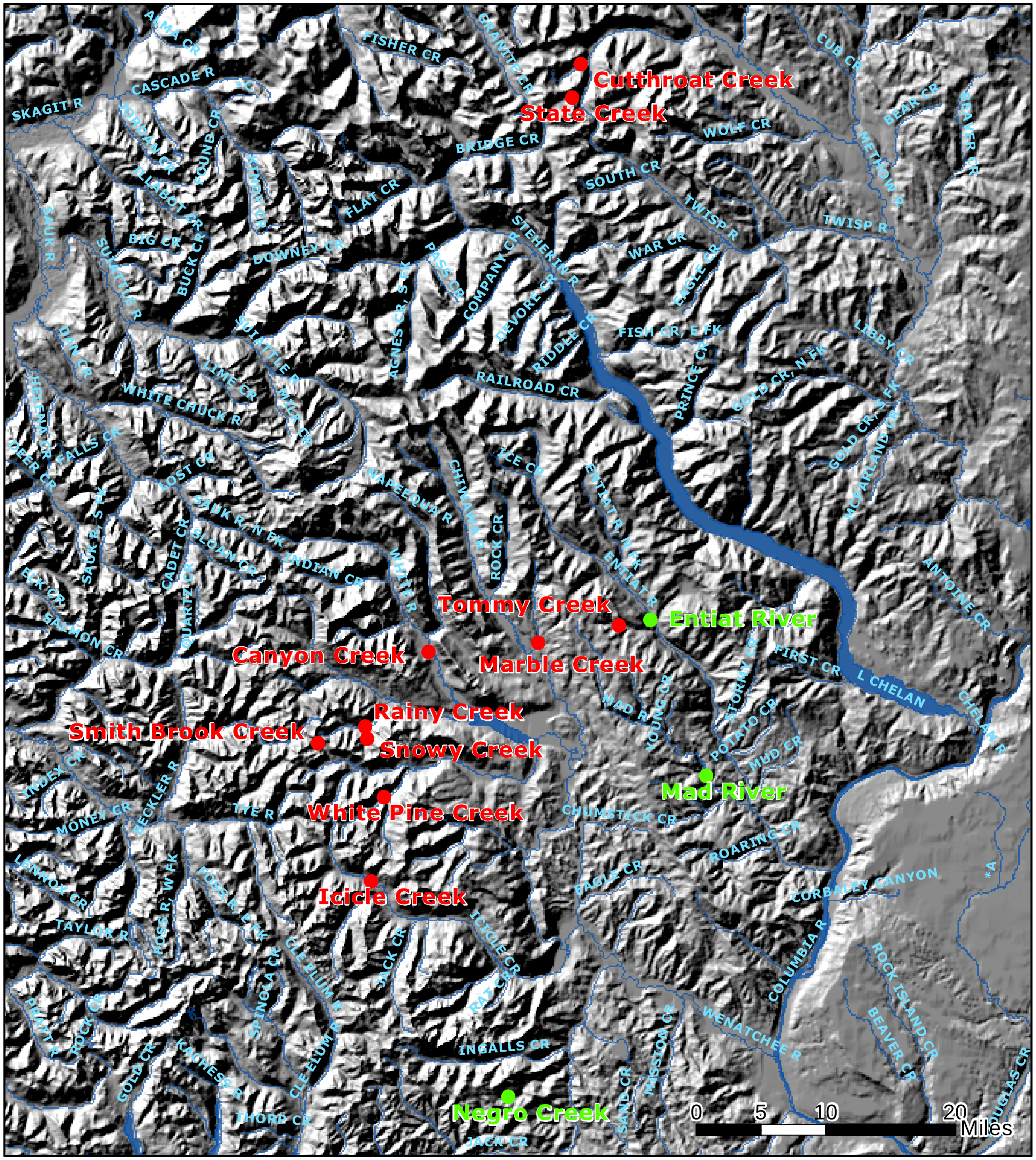
METHODS

Selection of Collection Sites

A list of thirty-two sites was compiled initially, with input from fisheries staff of the Wenatchee National Forest, focusing this year on locations where westslope cutthroat populations were believed most likely to be found. From these, we made collections and did photo-documentation at nine sites and collaborated with Forest Service personnel (we did the photo-documentation, they collected the genetic samples) at four additional sites. The locations of these sites are identified and mapped in Figure 1. The other sites were dropped, owing to problems of access or because, upon scouting, they turned out to be too small to yield to our collection method. Ten of the thirteen sites finally chosen were initially thought to be populated by westslope cutthroat trout and three with rainbow trout, but in the end four sites turned out to be predominately rainbow trout sites. We also added data from a fourteenth site (upper Icicle Creek in the Wenatchee River drainage, also a rainbow trout site) which we had collected in FY-98. The location of the Icicle Creek site is also shown in Figure 1.

In order to achieve even nine collections of westslope cutthroat in FY-2001, we made a major compromise in our preferred site selection protocol. Six of our collection sites were from headwater streams that included at least one lake in the headwaters or with an outlet nearby. All of these lakes had stocking histories of Twin Lakes strain westslope cutthroat. Ordinarily, we specify that there be no lakes in the headwaters of the drainages from which we collect, in order to avoid this possible source of hatchery origin fish that could confound our genetic analysis. The Washington Department of Fish and Wildlife propagates the Twin Lakes strain of westslope cutthroat trout and stocks these fish widely in high lakes of the region (Crawford 1979, 1998). Even though great pains are taken not to stock lakes where the fish might escape to reproduce downstream, such escapes occur rather commonly nevertheless, so we have always tried to avoid streams with lakes in their

Figure 1
2001 Sample Site Locations In the Wenatchee, Entiat, And Methow River Basins



Legend

- Sites With DNA Data
- Sites Without DNA Data
- Rivers

W A S H I N G T O N

T R O U T

headwaters. However, recent findings by Shaklee and Young (2000, see also Shaklee et al. in press 2002), who developed a more sophisticated method than ours to investigate genetic integrity of westslope cutthroat populations, indicate that despite past stocking, introgression with hatchery origin westslope cutthroat trout had not taken place in seven out of eight tributary populations examined in the Pend Oreille drainage. We reasoned that perhaps hatchery stocks introduced in our study area fared no better against indigenous populations than they did in the Pend Oreille region, and therefore it is important to document whether or not pure westslope cutthroat populations are present despite past stocking histories. So we went ahead with collections at several sites we would have otherwise avoided. Now that methodologies for doing so are in hand for westslope cutthroat trout, verification of native or hatchery origin at these sites can be left for some later study.

Stocking History of Collection Streams

Since 1932, when the State legislature vested all responsibility for fish and wildlife management in what is now the Washington Department of Fish and Wildlife (WDFW), the stocking of hatchery-reared trout in State waters has been a bread-and-butter practice of that agency. We examined two separate WDFW data bases for study area streams dating from 1932 to the present. One of these is the official record of WDFW fish stocking activities which is now maintained on electronic files at the Olympia office. The other data set was compiled for the Department separately to show where in the State hatchery-origin westslope cutthroat trout have been stocked (see Crawford 1998). We found a few discrepancies between these two data bases, and when we did, we relied on the "official" data base as the standard.

Prior to 1932, many other agencies and entities, including predecessor State agencies, the old U. S. Bureau of Fisheries, county fish and game agencies, and even individuals, also stocked trout in state waters. Unfortunately, no neat institutional history exists for any of these activities. We canvassed the archives of the Washington Department of Fish and Wildlife, all of the old State Fish Commissioner's reports that we could locate (Washington State Fish Commissioner 1905 through 1919, covering the period 1903 through 1918), and any other sources that came to our attention (e.g., Varley 1979) for whatever records might exist of trout-stocking activities in our collection streams and nearby waters. Although we cannot vouch for the completeness of these archives—and thus, can never be completely certain that the absence of a record for a given stream means no stocking ever occurred there—we nevertheless took the absence of a record as evidence that the population we found was native and untainted by stocking unless our genetic results indicated otherwise.

Based on available records and reports, we concluded that hatchery origin or non-native fish most likely to be encountered in the course of our work would include coastal rainbow trout *Oncorhynchus mykiss irideus*, coastal cutthroat trout *O. clarki clarki*, Yellowstone cutthroat trout *O. clarki bouvieri* (imported into the state in the past under the name "Montana black-spotted trout") and the Twin Lakes strain of westslope cutthroat trout mentioned above. Past shipments of "Montana black-spotted trout" into the state may have also included westslope cutthroat trout. According to Crawford (1979), all of Washington's hatchery rainbow broodstocks are derived from *irideus*, the coastal rainbow subspecies. We did learn during our FY-99 collections that WDFW has developed a broodstock of *Oncorhynchus mykiss gairdneri*, the inland rainbow subspecies, which is held at Phelan Lake north of Colville in Stevens County. However, we found no evidence from the stocking records that these fish have been stocked in our FY-2001 study area.

Other Recent Genetic Studies of Populations in the Study Area

The U. S. Fish and Wildlife Service has made several collections of resident trout populations in the study area, including some of the same streams where we did our work (Proebstel and Noble 1994; Proebstel et al. 1996; Proebstel 1998). We were also made aware of a more recent genetic study of trout populations of the Stehekin River drainage in North Cascades National Park, done for the National Park Service by a unit of the U. S. Geological Survey (Ostberg and Rodriguez 2002). We reviewed the results of these studies and incorporated pertinent findings in this report. Also, simultaneously with our field work, the U. S. Forest Service had a crew in the field making collections for a genetic analysis of its own. As noted above, we collaborated with this Forest Service crew, doing photo-documentation and assisting with fish collections at some of their sites in exchange for their genetic results for sites of interest to us.

Fish Collection and Work-Up

In the field, upon arrival at a collection stream, we first prospected for a convenient work-up site where we could set up our aquarium and other gear. We recorded the township, range, and section coordinates of this site from the appropriate USGS 7-1/2’ quadrangle map, and also its GPS coordinates read from a Garmin II-Plus unit. We also photographed the site and recorded its altitude and stream order (Strahler 1957) as determined from the map. When our equipment was set up, we deployed upstream and down from the work-up site to collect fish. We seldom had to cover more than 1.2 km (3/4 mile) of stream to collect all the fish we needed.

We collected all fish specimens by hook and line angling using artificial flies with barbless hooks. Non-target fishes were released straight away, although we kept track of the number captured. When a fish of a target species (cutthroat trout, rainbow trout and hybrids of the two) was brought to hand, we removed the fly and quickly placed the fish in a bucket of clean stream water, which itself was kept in the shade. We exchanged the holding water frequently to keep the fish cool and well-aerated. After 30-45 minutes of angling, we brought the fish to the work-up site, regardless of how many had been captured. If more fish were needed to complete our collection after the initial batch had been processed and released, additional 30-45 minute angling periods were employed. We recorded the total time spent angling to capture the requisite number of fish of the target species from each site, and from that calculated a catch per unit of effort (CPUE) value which we used as a surrogate for abundance of target species at the site. In the field, we recorded fish as either cutthroat trout, rainbow trout, or hybrid on the basis of visual inspection. We corrected these field calls later, when necessary, after the results of the genetic tests were in hand.

Fish were anesthetized individually or in groups of two or three, using the procedure described below. Each fish was then measured (fork length to the nearest mm) on a measuring board, weighed (wet weight to the nearest gram) using calibrated Pesola precision spring scales, and the adipose fin (or, on fish smaller than about 76 mm, a small snippet from the lower tip of the caudal fin) was removed with sharp, clean, stainless-steel scissors. These fin-tissue snippets were carefully placed in individual pre-labeled vials of preservative and saved for later use in the genetic analysis. The fish were then placed either in a bucket of clean stream water, or in a still but not stagnant part of the stream itself to recover from the anesthetic prior to release.

Anesthetic Protocol

We used clove oil (Anderson et al. 1997; Preiser et al. 1997; Keene et al. 1998) at 50 mg/L as our anesthetic in this work. During our FY-98 field work (see Trotter et al. 1999), we found that clove oil produces the same levels of anesthesia on about the same timetable, and recovery times are also about the same, as MS-222 at equal concentrations. Plus, clove oil carries a GRAS (Generally Recognized As Safe) rating from the U. S. Food and Drug Administration whereas MS-222 must be used with a 21-day withdrawal period before the fish can become fodder for human consumption. This can be an important consideration when collecting from streams open to recreational angling, as most of our sites were.

Clove oil is not completely soluble in water, and must first be dissolved in ethanol. We prepared stock solutions consisting of 3 mL of clove oil (density approximately 1 g/mL) made up to 30 mL with denatured 95-percent ethanol. Three-mL quantities of this stock solution were measured out into individual ethanol-proof capped vials which were kept in the dark in a refrigerator until taken into the field. The contents of one vial dispersed in 6 L of stream water in a 18.9 L (5 gal.) bucket gave us our 50 mg/L field concentration.

Fish Photography

Five to eight fish (most often six) from each collection site were not anesthetized immediately, but were placed individually in a small portable aquarium through which stream water was flowing, allowed time to acclimatize, and then photographed. Following its photo session, each fish was removed from the aquarium, anesthetized, and worked up as described above while the next fish was becoming acclimatized to the aquarium.

The aquarium used in this work is a portable “photarium” unit of a type originally described by Rinne and Jakle (1981). The unit is made of Plexiglas and measures 356 mm (14 inches) wide by 203 mm (8 inches) high by 51 mm (2 inches) deep. A small submersible pump (Teel model 1P811A) powered by a 12-volt gel-cell battery circulates stream water through the unit, thus maintaining an environment similar in temperature and oxygen content to the fish’s natural habitat.

The current in the tank induces the fish to assume a natural position without undue stress, enabling high quality color photographs to be obtained that are useful for documentation, taxonomic studies, and publication.

We photographed under direct natural lighting, with the light impinging on the front of the aquarium to minimize glare and reflections (midday lighting in bright sunlight, say between 10 AM and 2 PM, works best but we could not always control the timing of our photo-shoots, nor the quality of the light). The aquarium itself was shifted and reoriented when necessary to eliminate shadows. We always placed a layer of clean gravel in the bottom of the aquarium (after first filling the tank with water to prevent scratching the Plexiglas) to avoid having the bottom of the unit show in our photographs. We always shot against a plain background which consisted of a colored backdrop cloth (fleece works best) stretched over a board. We experimented with various background colors, but settled on forest green for photographing in strong direct light, and light blue for photographing under cloudy or mixed-light conditions. Figure 2 shows the assembled unit in operation at streamside.

To minimize loss due to camera malfunction, unforeseen loss of film, or accidents in processing, we shot two sets of photographs, a primary set and a backup set, of each fish. For the primary set, we used a Minolta Maxxum 600si camera equipped with autofocus and autoexposure features. We spot-metered exposures directly off the side of the fish, then used the camera's bracketing program to bracket the metered readings by ± 0.5 ev unit. All images in the primary photo set were made on 35-mm Kodachrome 200 transparency film, and all film was processed by the Kodak laboratory in Tukwila, Washington. The backup photo set was taken with a Nikon FE camera equipped with a 55 mm F 3.5 Micro-Nikkor lens. Backup images were exposed on Ektachrome EV100VS film at 100 ASA. We also experimented this year with a digital camera, a technique which shows great promise for this kind of work.

Because of the tradition for taxonomic measurements to be made on the left side of the fish (Behnke 1992), we photographed all fish facing left.

Figure 2

A photo catalog of our collections was prepared by first selecting the best images (one each) of each fish. These images were scanned and displayed individually on a computer and edited using Adobe Photoshop 5.5 for the Macintosh, then converted to page format using Adobe Pagemaker 6.5 for the Macintosh. The catalog is included as Appendix 1 of this Report.

Fin-Tissue Collection and Preservation

As noted above, we removed the adipose fin (or occasionally a caudal fin snippet of about the same size) from each fish collected, attempting at all times to retain the fin-clip on the scissors and avoid touching it with our fingers. We found this easiest to do if one of us held the fish and gently arched the back, thus presenting the adipose fin, while another person clipped the fin at the base, always approaching with the scissors from behind the fin. The fin-clip, now on the scissor tips, was then quickly transferred into a 2-mL vial filled with denatured 95-percent ethanol (Shiozawa et al. 1992). We used capped cryo-storage vials for this purpose, which had been pre-filled with ethanol and labeled with the site code and specimen number. We kept the tissue vials in a Coleman cooler while still in the field, and stored them in a home freezer at –20^{o} C until they could be transported to the genetic laboratory for analysis.

Collection Site Physical Description and Habitat Data

After completing our protocols for measuring, weighing, tissue collection and fish photography, we then recorded some basic measurements and observations of stream, riparian, and upland habitat condition at each collection site. Photographs were taken of the site at the outset, and subsequent photos were taken to pictorially record significant habitat features of each stream. One of these photos was chosen for each collection site to accompany the fish photos displayed in the appendix. We measured water temperature with hand-held thermometers at several times over the course of the day and recorded the range. Gradient was measured with a Peco hand-held Abney level on one or more stream sections chosen as being typical of the overall collection reach. We also attempted to determine stream discharge from measurements of water velocity and wetted channel width and depth, but then discovered that we had a malfunctioning flow meter, so these measurements were not included in the report.

In order to further describe the sites as we found them, qualitative rankings of fourteen additional habitat parameters relating to riparian vegetation, streambank condition, bottom substrate, and channel condition were made by visual estimation using a three-page Habitat Assessment Field Data Form, originally developed for research on aquatic oligochaetes and other aquatic invertebrates by Dr. D. Kathman, Aquatic Resources Center, Franklin, Tennessee (for a copy of the Habitat Assessment Field Data Form, please refer to Trotter et al. 1999). Each habitat parameter (for both left and right banks where appropriate) was evaluated from a choice of four comparative values, and each of the four values was given a numerical score within a five point range: Poor (1-5), Marginal

(6-10), Suboptimal (11-15), and Optimal (16-20). The maximum possible total score for a site was 360 points. Although subjective, this system was rapid and easy to use in the field, and provided a numerical means to compare individual habitat parameters among collection sites, and also to compare cumulative scores for each collection site. For consistency, the same person completed the ranking for all sites.

Calculation of Fish Condition Index

Condition indices are widely used to assess robustness or physiological well-being of fishes. We chose to calculate Relative Weight, $\mathbf{W_r}$ (Wege and Anderson 1978) as our measure of condition index due to its freedom from length-related and species-related biases (Cone 1989; Murphy et al. 1991). $\mathbf{W_r}$ is given by the formula:

$$\mathbf{W_r = (W/W_s) \times 100} \qquad (1)$$

where $\mathbf{W}$ is the weight of each individual fish (in grams) and $\mathbf{W_s}$ is a length-specific standard weight which is computed from one of these equations (Kruse and Hubert 1997; Simkins and Hubert 1996):

For interior cutthroat trout (lotic populations) $\mathbf{\log_{10}W_s = -5.189 + 3.099 \log_{10}TL}$ (2)

For rainbow trout (lotic populations) $\mathbf{\log_{10}W_s = -5.023 + 3.024 \log_{10}TL}$ (3)

where $\mathbf{TL}$ is total length (in mm).

In equations (2) and (3), length is specified as total length, $\mathbf{TL}$. Since we recorded fork length, $\mathbf{FL}$, in the field, we converted using these formulae:

For interior cutthroat trout (Kruse and Hubert 1997) $\mathbf{TL = (FL + 1.850)/0.977}$ (4)

For rainbow trout (Simpkins and Hubert 1996)) $\mathbf{TL = -0.027 + 1.072FL}$ (5)

Thus, to compute $\mathbf{W_r}$ from our field data, we applied equations (2) and (4) to the cutthroat trout specimens and equations (3) and (5) to the rainbow trout specimens to first compute $\mathbf{W_s}$ for each fish, then we plugged those values into equation (1) to compute the respective $\mathbf{W_r}$ values.

$\mathbf{W_r}$ was calculated for each individual based on its DNA results. Introgressed individuals that had 50 percent or fewer rainbow trout diagnostic markers were analyzed as cutthroat; individuals with more than 50 percent rainbow bands were analyzed as rainbow trout.

Statistical Analysis

Several statistical procedures were run on the collected data to screen our sampling methods for bias and to test for differences in mean condition index among sampled populations. Statistical analyses were performed using the NCSS 2000 statistical software package (Hintze 1999).

Because the Relative Weight index is dimensionless and is expressed as a proportion or percentage (= proportion *100), the W_r's cannot be expected to be normally distributed, nor can the variances of the samples be expected to be equal. W_r expresses fish condition independent of fish size (weight, length); for example, resident westslope cutthroat of 120 mm and 250 mm fork-lengths may both have a W_r of 90 (0.90). Hence, even if the original length distributions are normal and all sample populations have equal variances, one should expect the W_r's of a randomly sampled local population to have a repulsed distribution, with more individual values clustered close to the population mean value than in a normal distribution. The distributions of sample proportions can often be rendered normal by arcsine transformation. However, since W_r can have values greater than 100 (proportion greater than 1.0), arcsine transformation could not be used to attempt to normalize the repulsed data or equalize the sample variances. This generally invalidates the use of Analysis of Variance, which strictly requires that the sample populations to be analyzed have normal distributions and that their variances be equal.

Nevertheless, several Anovas were run on the W_r data to compare sample means, since the F-test is robust to mild violations of the normality and equality of variance requirements, provided the groups analyzed are random samples from their respective populations. Viewed in conjunction with Box Plots of the W_r data (Figure 4) and with the above caveats in mind, the analyses present a fairly reliable picture of the data.

The following tests were run on the W_r data. A One-Way Analysis of Variance on sample means was run to test for difference among the mean relative weights of the sampled populations. Because our collections spanned the entire summer season, we also conducted a Nested Anova using the NCSS GLM Anova tool with bi-weekly period of sampling as the fixed factor and stream sample population as the nested factor to test for the influence of collection date on differences in sample populations mean W_r values.

We also tested whether W_r values were correlated with length, since a strong correlation would indicate bias in the standard weight formula (W_s), bias in sampling method, or an unexpected length-related causal condition. Least squares regressions were run on each sampled population with individual W_r values as the dependent variable and individual length values as the independent variable, and scatter plots with the least square regression line through them were produced and inspected. Under ideal conditions and random sampling, there should be no correlation between W_r and length, and the slope of the regression line should be zero.

Finally, even though our collection site habitat quality scores (see above) were only qualitative, we tested for the relationship between population mean condition index and collection site habitat quality with linear (least squares) regression and scatter plots, as we did for W_r and length.

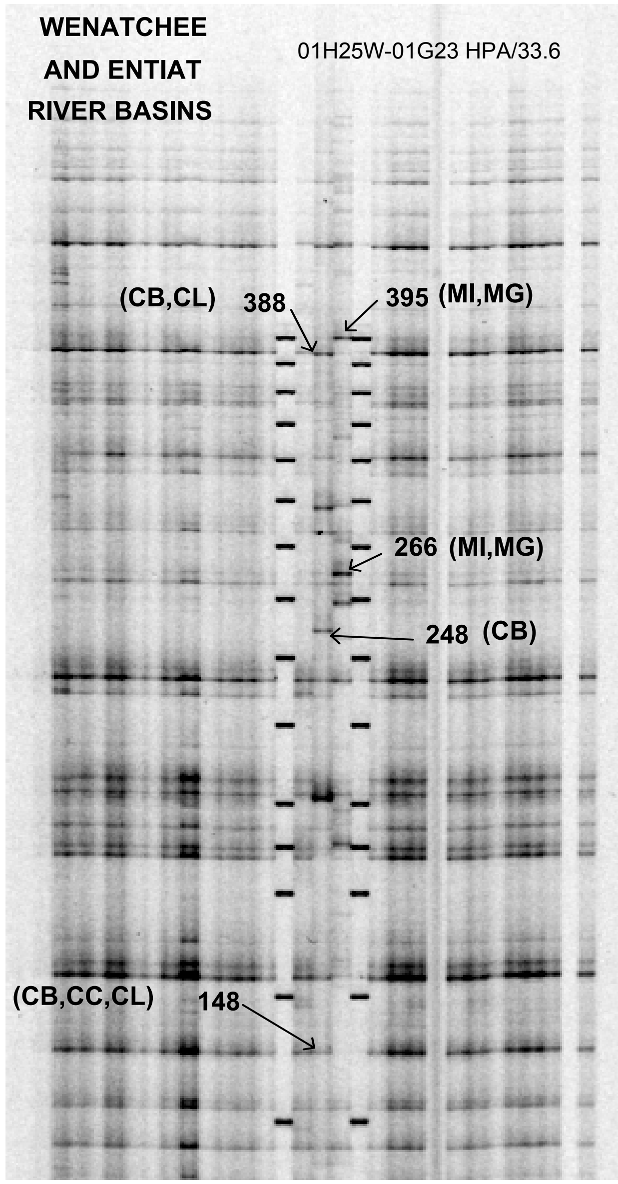
Genetic Analysis

We used paired interspersed nuclear elements (PINEs) to identify species and subspecies of the fish collected, and to assess the extent of hybridization that might have occurred in the populations (Spruell et al. 2001; Weigel et al. 2002 in press)). PINEs use pairs of primers that are complementary to the sequences of elements that are interspersed throughout the nuclear genome. Using the polymerase chain reaction (PCR), the fragments of DNA between these elements are amplified. When these amplified fragments are run on an electrophoretic gel, it is possible to reliably distinguish species based on the presence or absence of diagnostic bands. We used markers amplified by the same primer pairs to differentiate between coastal, Yellowstone, and westslope cutthroat trout and between coastal and inland rainbow trout.

With regard to hybridization between rainbow and cutthroat trout, PINEs do not always allow a distinction between inland and coastal forms of the rainbow trout component. This is because there are shared bands between the forms, and which of these bands will be expressed in the hybrid is random. Therefore, when levels of hybridization with rainbow trout are low, we cannot assign the hybrid influence to either form with certainty.

Fin clips stored in 95 percent ethanol, as described above, were transported to the University of Montana where DNA was extracted using guidelines provided with the Puregene™ DNA Isolation Kit. DNA was amplified using primers labeled with fluorescent dyes to allow visualization of the product. Each population was analyzed using a minimum of three primer pairs. PCR reagent volume was maintained at 10 μL. Reactions contained the following: approximately 25 ng of genomic DNA, 1 μL 10X Perkin-Elmer PCR buffer, 4.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5.0 pmoles of primer, and 0.5 U Stoffel Taq. Reactions were completed in a MJ Research PTC-100 thermal cycler. All reactions except those including the primer 33.6+2 used the following profile: 3 minutes at 95°C, 30 cycles of: 1 minute at 93°C, 1 minute at 60°C, 2.5 minutes at 72°C, and finally an additional 2.5 minutes at 72°C. For reactions that included the primer 33.6+2, the 60° annealing temperature was increased to 61°. Products were then refrigerated until analysis on an electrophoretic gel. Amplified products were run on a 4.5% polyacrylamide gel for 50-75 minutes at 65 watts. DNA products were then visualized using a Hitachi FMBIO-100™ fluorescent imager.

Figure 3. Example of a PINE using primers Hpa5 and Jeffreys 33.6. The analyzed population in Tommy Creek (code H) which exhibited no evidence of hybridization



Each gel was visually inspected to identify DNA fragments that were diagnostic for interior or coastal rainbow trout, or for westslope, Yellowstone, or coastal cutthroat trout. The size of these bands was confirmed using MapMarker LOW size standard and FMBIO software. All gels also included at least one known individual from each species and subspecies in question to be used as a reference for the unknown samples. An example of a PINE gel is shown in Figure 3.

Genetic Purity Rating

As we did in our FY-98 and FY-99 work (Trotter et al. 1999, 2000), we assigned genetic purity ratings to each of the collected populations using the following modification of an approach originally developed by Binns (1977) for cutthroat trout populations in Wyoming:

A. Pure stock. All individuals examined carry only markers of the species or subspecies of interest, and there is no history of stocking the water with hatchery fish of the same species or subspecies.

B. 1-9 percent of individuals examined carry bands from another species or subspecies, but appearance-wise, all are "good" representatives of the species or subspecies of interest. Also applied to populations with no detectable hybridization, but where a history exists of stocking the water with hatchery fish of the same species or subspecies.

C. 10-19 percent of the individuals examined carry bands from another species or subspecies, but all are still "good" visual representatives of the species or subspecies of interest.

D. 20 percent or more of the individuals examined carry bands from another species or subspecies, but all are still "good" visual representatives of the species or subspecies of interest.

E. A population never examined by a taxonomist or by any genetic method.

F. 20 percent or more of the individuals examined carry bands from another species or subspecies, and the specimens are questionable to poor visual representatives of the species or subspecies of interest. This designation would also apply to populations that are hybrid swarms.

As originally promulgated (Binns 1977), a lower purity rating was assigned if the stream from which the specimens came had any kind of a stocking history. Because we can easily detect foreign DNA bands in the specimens we examine, we determined that there was no need to downgrade purity based on stocking history alone if the only record of stocking was with a different but hybridizable species or subspecies, e.g., rainbow trout stocked in a cutthroat trout stream, or Yellowstone cutthroat stocked in a westslope cutthroat stream. However, we did downgrade based on stocking history alone if: (1) the record showed that non-indigenous fish of the same species or subspecies had been stocked, e.g., Twin Lakes strain westslope cutthroat stocked in a stream where we found pure westslope cutthroat trout; or (2) the stocked fish were the same species but were not identified as to subspecies or origin, e.g., fish identified only as “cutthroat trout” stocked in a stream where we found pure westslope cutthroat. We note here that in future studies of genetic purity, it may be possible to dispense with even this constraint. Newer, more advanced and detailed methodologies, albeit more costly and requiring more specimens per population, are now available which, among westslope cutthroat populations at least, appear able to distinguish between indigenous populations and those derived from hatchery stocks (see Shaklee and Young 2000; Shaklee et al. 2002 in press). Future studies should employ these methods to further examine populations designated B-purity on the basis of westslope stocking history alone.

In scoring, we rounded percentages to the nearest whole number. For example, if 9.1 percent of the individuals in a population carried foreign bands, we scored it 9 percent. If 9.5 percent of the individuals carried foreign bands, we scored it 10 percent.

RESULTS

General Characteristics of Collection Sites

Site coordinates and elevations, reach physical measurements, and habitat scores of our FY-2001 collection sites are given in Table 1.

Table 1. Coordinates and Stream Reach Data for Collection Sites
ND indicates no data.

Stream Name	Map Coordinates	GPS Coordinates	Reach Altitude	Stream Order	Reach Gradient	Water Temp. Start-end	Habitat Score
Canyon Creek A	28N 16E s19	47° 54.30N 120° 53.72W	610m	2	5	8 C 9 C	263
Canyon Creek B	28N 16E s19	ND	650m	2	5	8 C 9 C	299
Rainy Creek	27N 16E s20	47° 49.28N 120° 59.96W	841m	3	3.5	8 C 10 C	266
Upper Smith Brook	27N 13E s25	47° 48.14N 121° 04.67W	1219m	1	<1	7 C 13 C	287
Upper Negro Creek	22N 17E s8	47° 24.48N 120° 45.59W	1210	2	3	8 C 8 C	284
Marble Creek	28N 17E 21/22	47° 54.94N 120° 42.78W	756m	2	ND	11 C 11 C	297
Snowy Creek	27N 15E s29	47° 48.45N 120° 59.78W	1085m	2	2	8 C 11 C	331
State Creek	35N 17E s19	48° 31.48N 120° 39.47W	1651m	1	<1	18 C 23 C	233
Tommy Creek	28N 18E s9	47° 56.21N 120° 34.73W	1207m	2	5.5	8 C 11 C	298
White Pine Creek	26N 15E s21/22	47° 44.55N 120° 58.09W	1006m	3	2.5	9 C 11 C	300
Cutthroat Creek	35N 19E s5/6	48° 33.73N 120° 38.60W	1293m	2	7.5	9C 11 C	304
Icicle Creek	25N 14E s12	ND	1006m	ND	ND	8 C 8 C	Not Scored
Upper Mad River	ND	ND	ND	ND	ND	ND	ND
Upper Entiat Site	ND	ND	ND	ND	ND	ND	ND
Lower Entiat Site	ND	ND	ND	ND	ND	ND	ND

Fish Abundance, Condition Indices, and Other Parameters of the Fish Collections

Table 2 lists the proportions of different fish species encountered at each collection site, along with CPUE of the target species and computed population condition indices.

Table 2. Proportion of principal species and other salmonids encountered, CPUE for target species, and relative weights, FY-2001 collections.

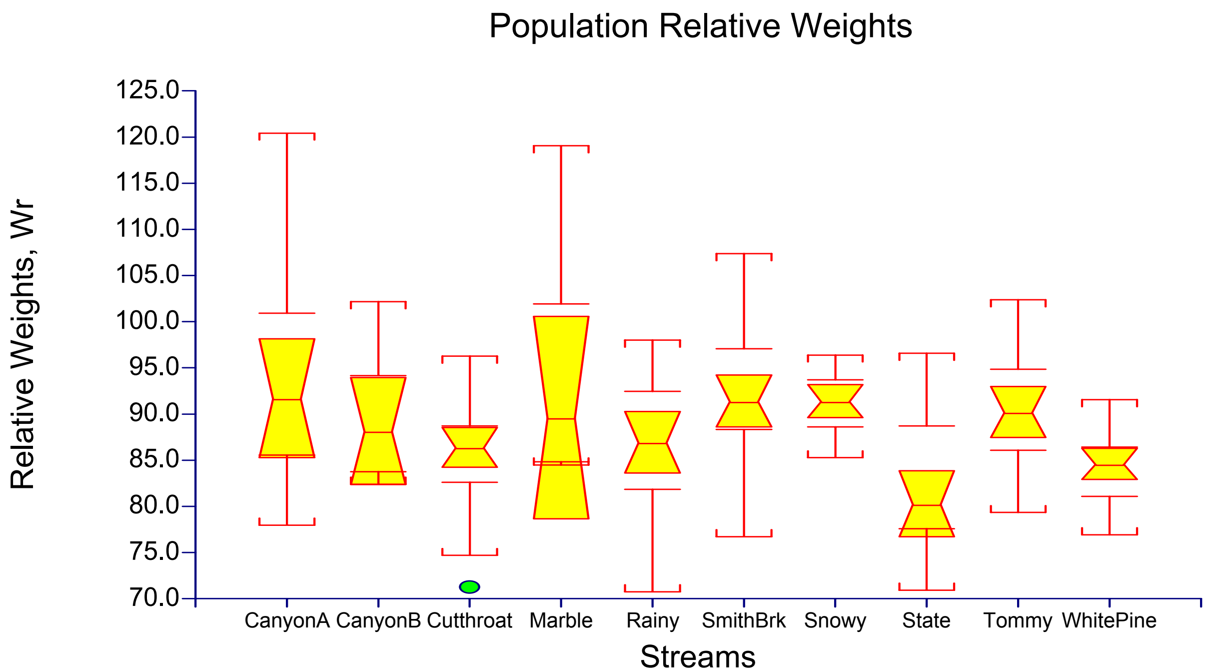
Collection Site	Date	Principal species	Other salmonids encountered	Target CPUE, fish/angler-hr	Population mean W_r
Canyon Creek A	6/27/01	WCT 81%	Hyb 19%	4.6	100
Canyon Creek B	6/27/01	WCT 89%	Hyb 11%		87
Rainy Creek	7/2/01	WCT 71%	Hyb 29.%	4.7	87
Upper Smith Brook	7/3/01	WCT 100%		3.7	93
Upper Negro Creek	7/10/01	WCT		6.7	
Marble Creek	7/12/01	WCT 100%		1.6	94
Snowy Creek	7/13/01	WCT 100%		2.5	91
State Creek	7/26/01	WCT 100%		7.1	82
Tommy Creek	8/16/01	WCT 100%		11.6	90
White Pine Creek	8/30/01	RB 84%	Hyb 18%	8.3	84
Cutthroat Creek	9/13/01	WCT 100%		16.0	85
Upper Icicle Creek	7/3/98	RB 100%		4.0	NA
Upper Mad River		RB	Bull trout		
Upper Entiat Site		RB			
Lower Entiat Site		RB	Bull trout		

As indicated in Table 2, westslope cutthroat trout is the principal species in nine of the collection streams. At six of the nine westslope cutthroat sites, populations are 100 percent westslope cutthroat; in the other two sites, the populations have been introgressed by rainbow trout. Results for Negro Creek, Upper Mad River, and the two Entiat sites had not been returned by press time. We had also expected to find westslope cutthroat trout as the principal species at the White Pine Creek site, but this one turned out to be rainbow trout in which 18 percent of the specimens also carry westslope cutthroat markers. The Upper Icicle Creek site collected in FY-98 is 100 percent rainbow trout. The Upper Mad, Upper Entiat, and Lower Entiat sites collected by USFS personnel are also rainbow trout sites. Counter to our experiences in FY-98 and especially in FY-99 in drainages where brook trout *Salvelinus fontinalis* had been widely stocked and were widely encountered, we did not encounter brook trout at any of our FY-2001 sites. Bull trout *Salvelinus confluentus* were encountered by USFS personnel at the Upper Mad River and Lower Entiat sites.

Most of our CPUE values were quite good and indicated high levels of target fish abundance at nearly all collection sites. We experienced a low CPUE (value less than 2 fish of the target species per angler-hour) at only one site, namely Marble Creek, a tributary of Chikamin Creek in the Chiwawa River drainage. We can offer no reason why the Marble Creek CPUE was so much lower than other sites.

The condition index of each population, expressed as mean relative weight Wr, is listed in the final column of Table 2 above. A more complete comparison of Wr values is presented in box plot form in Figure 4. In the figure, the line through the middle of each notched box is the median value of Wr for each population. Non-overlapping notched boxes have significantly different medians. The State Creek population, residing at the highest elevation, most exposed site and having the warmest water temperatures on the day of collection, had the lowest population Wr. The three populations with the smallest sample sizes (Canyon Creek A and B and Marble Creek) had the widest ranges of individual Wr values.

FIGURE 4



Statistical Analyses of Population Sample Data

Analysis of Variance conducted on the relative weights (Wr) of the ten populations for which DNA results have been obtained showed significant differences in the mean Wr between several populations, generally confirming the impression provided by the Box Plots of the same data (Figure 4). Scheffe's Multiple-Comparison Test singled out State Creek (lowest mean Wr score) as significantly different from Canyon A and Smith Brook at the alpha = 0.05 level. Canyon A was singled out as significantly different (at alpha = 0.05) from the streams with the four lowest mean Wr scores: Cutthroat, Rainy, State, and White Pine creeks. The remaining five populations (Canyon B, Marble, Smith Brook, Snowy, and Tommy) form a group with mid-range Wr scores that are not significantly different from one another or from Canyon A and the group with the four lowest scores. Three of the 10 stream populations (Canyon A, Canyon B, and Marble) had sample sizes lower than 20 resulting in significantly wider interquartile ranges (box lengths in Figure 4), and confidence intervals on the mean Wr value than the remaining seven populations that had sample sizes of 20 to 25. Canyon A had a sample size of 16, but a significantly wider interquartile range and confidence interval on the mean than the streams with only slightly larger sample sizes due to the presence of one exceptionally large individual of 340 mm total length with a Wr of 77.95. This Wr value was the lowest score within this population and a full 7 points lower than the next lowest score within this population.

Bi-weekly sample period had no detectable effect on population mean Wr score, though as expected with the relatively small sample size and number of degrees of freedom, power was low. Anova with stream population nested within bi-weekly sampling period, F-Ratio: 1.27, df (degrees of freedom) 4, $P = 0.39$, Power = 0.19 at alpha = 0.05.

Regressions of Wr values on total length for each population were not significant (regression slopes not significantly different from zero). Of the eight populations with sample sizes greater than 15 (i.e., not including Canyon B and Marble), slopes ranged from -0.152 (Canyon A) to 0.044 (Rainy), none of which was statistically significant. R-squared values ranged from 0.004 to 0.20. When the aggregate data set was considered (with the 340 mm individual from Canyon A excluded, as it was over 100 mm larger than any other fish collected), there was a significant negative slope (-0.10, $P = 0.0000$. Power = 0.999 at alpha = 0.05; R-square = 0.120), indicating that larger (longer) fish tended to be in poorer condition than smaller fish.

Statistical analysis of the total length data for each of the seven populations with sample sizes of 20 or more was unable to reject the null hypothesis that the sample lengths were normally distributed. However, visual inspection of length histograms and associated normal probability plots suggests that all seven samples tend to be more repulsed (platykurtic) than a normal distribution, indicating that our samples were drawn in a representative manner from across the range of expected individual sizes (lengths). Analysis of the distribution of total lengths for the aggregate data set (again, with the exceptional 340mm individual from the Canyon Creek A site excluded) did reject the null hypothesis of normality due to negative Kurtosis (D'Agostino Kurtosis: -3.1664, $P = 0.0015$; D'Agostino Omnibus: 10.0776, $P = 0.0065$), confirming the impression from the plots of seven individual populations.

Consideration of the range of sizes (total lengths) supports the impression that our sampling was representative across the range of sizes of the populations samples. Ranges of sampled total lengths for each of the populations were as follows: Canyon A: (110, 182 -- excluding the 340mm individual), Canyon B: (101, 181), Cutthroat: (105, 189), Marble: (102, 204), Rainy: (136, 238), Smith-Brook: (97, 189), Snowy: (104, 217), State: (133, 219), Tommy: (88, 220), White Pine: (121, 222).

Finally, habitat condition as measured and scored by our qualitative survey was uncorrelated with mean population Wr. Regression of mean Wr on habitat score: slope - 0.022 (ns), R-square - 0.012.

Photo Catalog of Trout Specimens

The photo catalog of live specimens representing each of the collected trout populations, along with a photograph of each respective collection site, is included in Appendix B.

Spotting Phenotypes

In FY-98, in our collections in the Yakima River basin, we recorded four distinct spotting patterns among that basin's westslope cutthroat trout (see Trotter et al. 1999 for illustrations and photographs of these four patterns). The westslope cutthroat trout in our current collections had nearly as much variability in spotting pattern. Only two populations, those of Canyon and Rainy creeks displayed the "classic" westslope spotting pattern, described by Behnke (1992) as small, irregular spots most thickly distributed on the posterior of the body, with no spots at all or only a very few within an arc extending from the origin of the pectoral fin to a point just above the lateral line and downward to the origin of the anal fin. Minimal to non-existent anterior spotting was present in the seven other populations sampled and it was the dominant pattern in Snowy, State, and Cutthroat creeks.

DNA Analysis

Results of the DNA analysis of collected specimens from each population are tabulated in Table 3. Shown here are the total number of specimens examined from each population, the number of genetically pure westslope cutthroat individuals, the number of genetically pure rainbow individuals, and the number of hybrid individuals found in each population.

Table 3. Genetically pure and hybridized individuals in FY-2001 collections
(ND signifies no data; results not available at press time)

Collection Site	Number of Specimens	Number of Cutthroat	Number of Rainbow	Number of Hybrids
Canyon Creek A	16	13	0	3
Canyon Creek B.	9	8	0	1
Rainy Creek.	24*	17	0	7
Upper Smith Brook.	23*	23	0	0
Upper Negro Creek	ND	ND	ND	ND
Marble Creek	5*	5	0	0
Snowy Creek.	20	20	0	0
State Creek	25	25	0	0
Tommy Creek	25	25	0	0
White Pine Creek	25	0	21	4
Cutthroat Creek	19*	19	0	0
Upper Icicle Creek	9	0	9	0
Upper Mad River	ND	ND	ND	ND

Upper Entiat site	ND	ND	ND	ND
Lower Entiat site	ND	ND	ND	ND

*One tissue sample did not amplify. Number of specimens = number samples collected minus one.

Six of our collection sites were free of hybrids and contained only cutthroat trout. One site was free of hybrids and contained only rainbow trout. Of the cutthroat subspecies tested for, only westslope cutthroat markers were found in collected specimens, be they pure specimens or hybrids. Unfortunately, we are not always able to clearly distinguish between interior and coastal rainbow trout. As explained in the Methods section, owing to shared bands between the two forms, the PINEs methodology does not always allow a clear distinction between the two. The Upper Icicle population appears to be pure interior rainbow trout, but we cannot be sure about the rainbow trout of White Pine Creek.

Stocking History of Collection Streams

We found stocking records for six of our eleven FY-2001 collection streams. Five streams—Canyon Creek, Snowy Creek, Marble Creek, State Creek, and Cutthroat Creek—had no stocking history of any kind that we could locate (a stocking record for Canyon Creek listed in Crawford (1998) was actually for a Canyon Creek in the upper Skagit system according to the official WDFW data base). However, one of the streams with no stocking record, namely Cutthroat Creek, has a lake in its headwaters which was stocked once (in 1967) with Twin Lakes strain hatchery westslope cutthroat trout; and the Marble Creek site lies just upstream of the confluence with Chikamin Creek, which does have an extensive stocking record and no barriers to bar migration between the two. The stocking record for a sixth stream, Tommy Creek, lists only a single stocking with rainbow trout (back in 1939), but this stream also has lakes in its headwaters which have been stocked extensively with both rainbow and Twin Lakes strain hatchery westslope cutthroat.

Our FY-98 collection site at approximately river km 46 (river mile 28.5) on upper Icicle Creek lies within the Alpine Lakes Wilderness and is accessible only by trail. Angling pressure here is relatively light. Downstream of the Wilderness boundary Icicle Creek is accessible by road, and several popular campgrounds are located along this downstream reach. From 1933 through 1993, the downstream reach was heavily stocked with hatchery rainbow trout for a popular recreational fishery. Most of this stocking occurred from river km 30 (river mile 18) downstream, and nothing in the record indicated any stocking of the creek ever extended upstream into the Wilderness. However, earlier unrecorded upstream stocking may have occurred, as anglers have reported capturing brook trout in the creek in the vicinity of our collection site, and the record does show that brook trout were stocked in Icicle Creek (location not recorded) in 1956. In addition, several lakes at the heads of tributaries upstream of our site, along with Josephine Lake at the head of Icicle Creek itself, have also been stocked many times over the years.

Genetic Purity Ratings

Tables 4 and 5 combine stocking histories with the results of our DNA analysis to yield the Binns genetic purity ratings for each of our FY-2001 trout collections. Table 4 shows the ratings for the nine cutthroat trout collections, and Table 5 shows the ratings for the five rainbow trout collections.

Table 4. Hybridization, summary of stocking history, and purity ratings for FY-2001 westslope cutthroat collections

(ND signifies no data; results not available at press time)

Collection Site	Percent Hybrids	Record of Stocking with CT (or RB)	Lakes Stocked with CT (or RB) in Headwaters?	Modified Binns Class
Canyon Creek A	19	No	No lakes; nearby White River stocked (RB)	C
Canyon Creek B	11	No		C
Rainy Creek	29	CT 32, 36, 42, 44, 45, 46, 48. (RB 37, 39-41)	Yes CT (and RB)	D
Upper Smith Brook	0	CT 37, 45, 55.	No	B
Upper Negro Creek	ND	CT 18. (RB 33, 38, 43, 44, 47)	No	ND

Marble Creek	0	No	Chikamin Creek, CT (and RB), no barriers	B
Snowy Creek	0	No	No	A
State Creek	0	No	No	A
Tommy Creek	0	No (RB 39)	Yes CT (and RB)	B
Cutthroat Creek	0	No	Yes, CT 67	B

We conclude that westslope cutthroat populations in Snowy Creek and State Creek merit A-ratings for genetic purity owing to freedom from hybridization and the absence of any record of past cutthroat stocking. These two populations are deemed the most likely to be native stocks untainted by contact with stocked rainbow trout or by interbreeding with stocked cutthroat trout from outside their respective basins.

Four additional populations, those in Upper Smith Brook, Marble Creek, Tommy Creek, and Cutthroat Creek, were given B-ratings. Even though these populations are genetically pure westslope cutthroat trout, records of cutthroat stocking of headwater lakes or nearby connected streams with no barriers to interchange exist for each. Therefore, interbreeding with non-indigenous westslope cutthroat stocks cannot be ruled out by our methods.

Canyon Creek sites A and B were both rated C-purity because 19 percent and 11 percent of individuals respectively were hybridized by rainbow trout, and the Rainy Creek population received a D-rating because of an even higher percentages of hybridized individuals. All fish in these populations, except one, were visually good representative of the westslope cutthroat phenotype. The one exception was Canyon Creek site A specimen A7, which we identified as a rainbow trout in the field. Subsequent genetic analysis indicated this fish was a hybrid with 67 percent of its markers derived from rainbow trout. The Canyon Creek populations pose something of a mystery in that no stocking record of any kind was found for this stream. Evidently rainbow trout (or steelhead) from the White River can access both Canyon Creek sites, even though site B is upstream and separated from Site A by a pair of falls that are each 1.8 to 2.4 m (6 to 8 ft) high. The presence in our collection from the lower (A) site of an individual of 340 mm that was over 100 mm larger than any other individual collected in any of the 10 streams and that had a significantly lower Wr score (77.95) than any other individual collected at that site is further evidence that many fish in this reach of Canyon Creek during June and early July are likely spawning fish from the White River.

Table 5. Hybridization, summary of stocking history, and purity ratings for FY-2001 rainbow trout collections
(ND signifies no data; results not available at press time)

Collection Site	Percent Hybrids	Record of Stocking with RB (or CT)	Lakes Stocked with RB (or CT) in Headwaters?	Modified Binns Class
White Pine Creek	18	RB 36, 39, 41, 42, 48 (CT 33, 34)	Yes (CT)	C
Upper Icicle Creek	0	No	Yes RB (and CT)	A
Upper Mad River	ND	ND	ND	ND
Upper Entiat Site	ND	ND	ND	ND
Lower Entiat Site	ND	ND	ND	ND

Our results indicate that the White Pine Creek site is inhabited by a C-purity rainbow trout population, probably resulting from the mixed stocking history that includes both cutthroat and rainbow trout. It is not possible to tell from our data what the original population of this reach of White Pine Creek might have been, but anecdotal information passed on by USFS suggests it was cutthroat trout.

Regarding the upper Icicle Creek population, all individuals in this collection had markers usually seen in interior rainbow trout and none had markers usually seen in coastal rainbow trout. Therefore, despite the ambiguity sometimes present in PINE results for rainbow trout, we conclude that the upper Icicle Creek population is a genetically pure A-population of interior rainbow trout.

Even so, some individuals did possess unique characters. Two individuals were missing a marker that was present in all other individuals in the collection, and these same two fish plus one other contained several bands that the lab had not seen before in any rainbow population, either interior or coastal.

Results of Other Genetic Investigations in the Study Area

Dr. R. J. Behnke of Colorado State University (cited in Brown 1984 at pp 109-111) performed what may be the first genetic analysis of trout specimens from this study area in 1982 when he examined several fish from Flat Creek, an upper Stehekin River tributary (Lake Chelan drainage). Based on meristic and morphological characters, Behnke pronounced these specimens “pure” westslope cutthroat trout. Prior to our collections, the work of Proebstel and Noble (1994), Proebstel et al. (1996) and Proebstel (1998) for the U. S. Fish and Wildlife Service has been the most extensive. These workers used a combination of meristics, morphology, allozyme electrophoresis, and mitochondrial DNA (mtDNA) analysis to examine specimens from many sites in the Wenatchee, Entiat, and Methow drainages. They classified populations as “pure,” “essentially pure,” “good,” “hybrid (C/R),” or “hybrid (R/C).” A population was “pure” if all characters were deemed within norms for the taxon; “essentially pure” if only one or two characters were outside their norms; “good” if most specimens were hybrids but were still visually good representatives of the taxon; and “hybrid” if not good visual representatives of the taxon. For obvious hybrids, (C/R) was attached if the fish had more cutthroat characters than rainbow or most resembled cutthroat trout visually, and (R/C) was attached if the outcome was the other way around. No account was taken of stocking history in any of this work. The Binns method, as we employed it in our evaluations, is a much more rigorous approach.

Even so, Proebstel and his co-workers recorded “pure” westslope cutthroat populations at seven locations, including our Snowy, Cutthroat, upper Negro, and Tommy Creek sites. They singled out the “pure” populations they found in Cutthroat Creek, nearby upper Early Winters Creek, and Robinson Creek, another upper Methow River tributary, as the best and most important examples of westslope cutthroat trout in the entire Methow drainage.

Proebstel and his co-workers also recorded “essentially pure” westslope cutthroat populations at 15 additional sites, among them Smith Brook, one of the streams where we collected; and visually “good” (although hybridized) populations at another 19 locations. They noted, however, that even in streams inhabited by “pure” or “essentially pure” populations, there was often a downstream graduation into a hybrid zone, and then often into a rainbow-only zone in the lowermost reaches or in the mainstems where the tributaries emptied.

Proebstel and his co-workers found “pure” or “essentially pure” rainbow trout which they designated as the interior subspecies *O. mykiss gairdneri*, at seven sites. These were upper Peshastin, Sand, and upper Icicle creeks in the Wenatchee River drainage; Roaring Creek in the Entiat drainage; and Goat, West Fork Buttermilk, and Little Bridge creeks all in the Methow River drainage.

Using microsatellite DNA markers, Ostberg and Rodriguez (2002) found two pure westslope cutthroat populations (in upper Park Creek and upper Stehekin River) and six cutthroat populations in which 5 percent to 34 percent of the individuals carried rainbow trout markers. They also found one rainbow trout population (in the mid-Stehekin River) in which 37 percent of the individuals carried westslope cutthroat markers, and one hybrid swarm (in the lower Stehekin River).

DISCUSSION

Native Range of Westslope Cutthroat Trout in Washington, and Other Observations on Collection Sites and Populations

Our discovery of two A–populations (no hybrids and no history of stocking) of westslope cutthroat trout in headwater tributaries of the Wenatchee River and Lake Chelan basins is significant because it lends additional support to the assertion that the original range of *O. c. lewisi* extends across the northeast corner of Washington State westward to the Cascade crest, then south along the east side of the crest to at least the South Fork of Toppenish Creek in the Yakima River basin. Westslope cutthroat trout in the John Day River system of Oregon (Behnke 1992, 2002) are likely a relict part of this original distribution. Evidence for this range extension has been building ever since Dr. R. J.

Behnke, the noted authority on western North American trouts, identified specimens sent him from Flat Creek, a Stehekin River tributary (Lake Chelan drainage), as pure westslope cutthroat trout in 1982 (see Brown 1984 at pages 109-111). Other support comes from reports and surveys published by Williams and Mullan (1992); Proebstel and Noble (1994); Proebstel et al. 1996; Proebstel 1998; Trotter et al. (1999, 2000); Williams (2000); and most recently, Shaklee and Young (2000) and Shaklee et al. (2002 in press).

These studies, along with our findings in the present study, also make it apparent that the evolutionarily younger and later-invading interior subspecies of rainbow trout *O. m. gairdneri* has not completely displaced native cutthroat from this range, especially not from the uppermost reaches of trout-bearing waters which may be below the minimum thermal tolerance of this form of rainbow trout. Mullan et al. (1992) set this minimum thermal tolerance level at about 1,600 annual temperature units (defined as the sum of average daily water temperatures above 0^{o} C over the entire year) for tributary streams in our study area.

Both A-populations of westslope cutthroat trout discovered in this study are found at sites with no lakes in their systems and where they could not have been reached in recent times by fish swimming from other stocked sites elsewhere. Snowy Creek, for example, is a high elevation tributary of Rainy Creek in the Little Wenatchee River system. The stream reach harboring its A-population is in a hanging valley formed by the passage of a Pleistocene-age alpine glacier that scoured away the side slopes of the Rainy Creek valley, leaving the collection reach suspended about 183 m (600 ft) above Rainy Creek and isolated from that stream by a very steep drop. The State Creek A-population is found in the uppermost headwater of that stream, which is part of the upper Stehekin River system in the Lake Chelan drainage. Several barrier falls isolate the State Creek A-population from any downstream influx.

We believe a case could also be made for upgrading two of our B-populations of westslope cutthroat trout, even though we do not do so here. These are upper Smith Brook (Wenatchee drainage) and Cutthroat Creek (Methow drainage). Both were rated B because of a history of stocking with hatchery cutthroat trout, either in the stream itself (Smith Brook) or a lake at its source (Cutthroat Creek). Our argument in favor of upper Smith Brook revolves around access for hatchery releases.

Locations of releases into Smith Brook are not specified in the record, but access is easiest in downstream reaches, which are also the most popular with anglers. These reaches are isolated from our collection reach by a series of 1.8 to 2.4 m (6 to 8 ft) vertical falls, plus at least one long, steep, high velocity bedrock sheet. Any one of these, but especially the bedrock sheet, would be a total barrier to upstream migration of hatchery origin fish. A precipitous tributary with a stocked lake at its source also enters Smith Brook downstream of these barriers, so any trout that survived the downstream drop from the lake to Smith Brook could still not move upstream to our collection reach.

With regard to Cutthroat Creek (and Cutthroat Lake at its source), names such as this are usually bestowed on a body of water based on the presence of fish. However, WDFW has consistently maintained that a lake or creek was fishless (especially those in the high Cascades where most of the lakes were indeed fishless owing to the presence of glaciers) until stocked by the Department with hatchery reared trout (see Crawford 1998 for example). In the cases of Cutthroat Creek and Cutthroat Lake, that stance is not supported by the agency's own records. The names Cutthroat Lake and Cutthroat Creek first came into use in 1917 (USDA Forest Service 1917), 54 years prior to the only record of stocking found in the WDFW data base, which occurred in Cutthroat Lake in 1967. Therefore, as unlikely as it might seem, the Cutthroat Creek/Lake system may have been a naturally fish-bearing system historically, perhaps recolonized from some downstream refugium after the retreat of the last Pleistocene ice sheet.

Westslope Cutthroat Appearance Phenotypes

Appearance-wise, we found the westslope cutthroat trout in our present collections to have nearly as much variability in spotting pattern as we found in the Yakima River drainage during our 1998 collections (Trotter et al. 1999). Only two populations, those of Canyon and Rainy creeks (both extensively hybridized) displayed the “classic” westslope spotting pattern.. Minimal to non-existent anterior spotting was present in the seven other populations sampled and it was the dominant pattern in Snowy, State, and Cutthroat creeks.

The cutthroat trout of Cutthroat Creek are the most vividly colored we have encountered. The vivid red bellies with the colors extending up the sides and between the parr marks of some specimens reminds us of the very colorful Colorado River cutthroat *O. c. pleuriticus* and greenback cutthroat subspecies *O. c. stomias* that one observes in the Rocky Mountain regions of Colorado.

Westslope Cutthroat Status and Management Considerations

Not long ago, Shepard et al. (1997) estimated that westslope cutthroat trout of at least 90 percent genetic purity presently inhabit less than 3 percent of the former range within the upper Missouri River drainage, which has long been regarded as the heart or core of this subspecies' historic distribution (Behnke 1992). Of 16 upper Missouri subbasins still supporting at least one population, 14 contain populations at moderate or high estimated risk of extinction, and almost all of these remaining populations exist in isolation from one another in high elevation mountainous stream fragments less than 10 km long. Shepard et al. (1997) identified existing and future land use activities and introduced species as the principal threats to these upper Missouri populations

If we apply this same 90 percent genetic purity criterion (which is less stringent than the Binns criteria we used in this study) to the populations we examined, then six of our populations qualify. We have no information on how many stream km each of these qualifying populations occupy, but the elevations of our collection sites for these six populations range from 756 m to 1651 m (2480 ft. to 5417 ft.) which is high elevation relative to the basin relief of the North Cascades Ecoregion (EPA 1995, 1998). Also, based on the work of Shaklee and Young (2000, see also Shaklee et al. 2002 in press), we can say that each of these populations exists as a reproductively isolated, separate stock.

So in these regards, it would appear that the high purity westslope cutthroat populations of our FY-2001 study area share the status of their upper Missouri conspecifics, and probably similar estimated risks of extinction as well. Our data provide little basis for commenting on the threats of existing and future land use activities to these populations. However, we can say that our study area populations do not appear to be as vulnerable to displacement by introduced brook trout as we found in our collections in the Yakima and Pend Oreille drainages (Trotter et al. 1999, 2000). Indeed, we did not encounter brook trout at any of our FY-2001 collection sites. The major potential threat in this study area appears to be introgression by hatchery origin rainbow trout which has, apparently, already made inroads in a number of westslope cutthroat populations.

Future Studies

It has been known for some years now that the westslope cutthroat trout subspecies is characterized by extreme genetic divergence among populations (Allendorf and Leary 1988). The recent work of Shaklee and Young (2000) and Shaklee et al. (2002 in press) confirms this for northeastern Washington populations, and furthermore, provides for the first time a nonlethal method for sorting out unique indigenous populations of this subspecies from those influenced or founded by hatchery releases. For example, WDFW has stocked the Twin Lakes strain of hatchery westslope cutthroat trout widely in our study area. We have identified two A-purity populations in this study, and other A-purity populations were identified in our Yakima basin report (Trotter et al. 1999).in streams where hatchery stocking evidently did not take place. But in both studies we also identified as many or more B-populations which were genetically pure westslope cutthroat, but the streams where we found them had been stocked in the past with hatchery cutthroat trout. Now, using the method of Shaklee et al., it should be possible to ascertain which of these populations were established via releases of hatchery stocks and which were not. We think it should be a high priority to go back in the field and resample all B-populations in east-central and northeastern Washington which were rated such because of stocking history, with this objective in mind. This additional insight into the origins of these stocks would be an invaluable addition to the known catalog of pure, indigenous populations of this subspecies in the mid- and upper Columbia River basin.

The A-purity State Creek population of westslope cutthroat trout offers a second important opportunity for meaningful future study. In this high elevation reach, which is at the very source of State Creek, we found an abundant westslope population effectively enduring water temperature fluctuations far more extreme than anything we had observed before, and indeed, anything we have found in the literature for this subspecies. We had previously documented no headwater westslope populations inhabiting streams with water temperatures above 15.6^{o} C (60^{o} F). In fact, the summer water temperature range we had typically found them in was 7 to 13^{o} C (45 to 55^{o} F). At the State Creek collection site (in a subalpine meadow some 732 m (2,400 ft) long situated near the crest of

Washington Pass), we found a westslope cutthroat population inhabiting water that measured 18^o C (64^o F) at 12:45 pm when we began our collection and 23^o C (74^o F) at 4:50 pm when we finished. The peak air temperature that afternoon in late July was not measured but was estimated at no more than 27^o C (80^o F). Of even greater interest, 366 m (1,200 ft) upstream from the site where these temperatures were recorded, at the northeast corner of the meadow, State Creek bubbles out of the ground with a constant spring temperature of 4.5^o C (40.5^o F)─a spread of 18^o C (33 F) from its source to our set-up site about halfway through the meadow. One of us (BM) had visited this meadow on two occasions earlier in the season (but in prior years) and had found the meadow covered with snow on the first visit (in early May) and partially flooded amidst patches of snow on the second visit (in mid-June). On that mid-June excursion, the water temperature approximately 100 m (300 ft) downstream from the spring was 4.5^o C (40^o F) and the trout were observed to be actively spawning. If these temperature extremes are typical it would afford an extreme opportunity for thermal regulation by this population during the mid-summer growing season to which this cutthroat population has likely adapted.

We believe that further focused study of the westslope cutthroat population at the headwaters of State Creek would provide valuable understanding of how such populations adapt to extreme habitat niches, and may even be a good test of the Mullan et al. (1992) concept of a minimum thermal tolerance level at about 1,600 annual temperature units for Washington's westslope cutthroat trout as a mechanism for resisting encroachment of invading or introduced trout.

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